IS1500, an IS3-like element from *Leptospira interrogans*

Caroline Boursaux-Eude,¹ Isabelle Saint Girons¹ and Richard Zuerner²

Author for correspondence: Richard Zuerner. Tel: +1 515 239 8392. Fax: +1 515 239 8458.
e-mail: rzuerner@asrr.arsusda.gov

¹ Unité de Bactériologie Moléculaire et Médicale, Institut Pasteur, 75724, Paris Cedex 15, France

² Leptospirosis and Mycobacteriosis Research Unit, National Animal Disease Center, Agricultural Research Service, US Department of Agriculture, PO Box 70, Ames, IA 50010, USA

Copies of an insertion-sequence (IS)-like element were isolated from two closely related serovars of *Leptospira interrogans sensu stricto*. Nucleotide sequence analysis of the 1236 bp element showed a characteristic IS structure with terminal imperfect inverted repeats (IRs) flanking a 1159 bp central region. This element was designated IS1500. Four open reading frames (*orfA*–*orfD*) were found in the central 'unique' region of IS1500. Similarities were detected between ORFA and ORFB and the putative transposases from members of the IS3 family of transposable elements. IS1500 or IS1500-like sequences were also detected in all other pathogenic leptospiral serovars, but not in the saprophytic species *L. biflexa*. Differences in IS1500 copy numbers in members of the same species suggest that this element can transpose. Physical mapping of IS1500 insertions in *L. interrogans* serovars *icterohaemorrhagiae* and *pomona* showed insertions were only on the large chromosomal replicon. The location of some IS1500 insertions coincides with regions of the genome that have undergone large rearrangements.

Keywords: insertion sequence, *Leptospira*, IS3-like

INTRODUCTION

Transposable elements are specific DNA sequences that can insert into non-homologous sites in various genomes. Insertion sequence (IS) elements are a class of transposable elements found in bacteria (Galas & Chandler, 1989). Typically, IS elements range in size from about 700 to 2500 bp and contain one or more open reading frames (ORFs). These ORFs usually encode proteins associated with transposition of the element. Inverse repeats (IRs) about 10–40 bp long are usually (but not always) found at the ends of IS elements (Galas & Chandler, 1989). Insertion of an IS at a new site often generates a small duplication of part of the target DNA. Most well-studied IS elements are found in the *Enterobacteriaceae*, and much of our knowledge of the mechanisms of transposition comes from these studies (Galas & Chandler, 1989). In recent years IS or IS-like elements have been isolated from nearly all evolutionary branches of bacteria, including spirochaetes (Pacciarini *et al.*, 1992; Saint Girons *et al.*,

1992; Woodward & Sullivan, 1991; Zuerner *et al.*, 1993a, b; Zuerner, 1994).

We are studying pathogenic spirochaetal bacteria in the genus *Leptospira*. Until recently all pathogenic members of the genus *Leptospira* were classified as *Leptospira interrogans sensu lato*. DNA homology studies showed the existence of extensive heterogeneity within *L. interrogans sensu lato* (Yasuda *et al.*, 1987; Ramadass *et al.*, 1992). These studies led to the reclassification of *L. interrogans sensu lato* into seven distinct species: *L. borgpetersenii*; *L. inadai*; *L. interrogans sensu stricto*; *L. kirschneri*; *L. noguchii*; *L. santarosai*; and *L. weilii* (Yasuda *et al.*, 1987; Ramadass *et al.*, 1992). There are no known mechanisms of genetic exchange between members of the genus *Leptospira*. Therefore, little is known about the genetics of these bacteria.

We recently showed evidence for gross alterations (inversions, deletions and insertions) in the genetic organization of two closely related serovars of *L. interrogans sensu stricto* (Zuerner *et al.*, 1993b). We hypothesized that recombination between like copies of repetitive DNA may be used to generate the rearrangements detected during physical mapping studies. Several different types of repetitive DNA, including IS-like elements, occur in the leptospiral genome (Pacciarini

Abbreviations: CFGE, continuous-field gel electrophoresis; IR, inverted repeat; IS, insertion sequence; PFGE, pulsed-field gel electrophoresis.

The GenBank accession numbers for the nucleotide and derived protein sequences reported in this paper are U13012 (IS1500A) and U13013 (IS1500B).

et al., 1992; Ralph & McClelland, 1993; Saint Girons *et al.*, 1992; Van Eys *et al.*, 1988; Woodward & Sullivan, 1991; Zuerner, 1993, 1994; Zuerner *et al.*, 1993a, b).

Studies of *L. borgpetersenii* provide insight into how IS-like elements contribute to the fluidity of the leptospiral genome. An IS-like element, IS1533, has been isolated from *L. borgpetersenii* and characterized (Woodward & Sullivan, 1991; Zuerner & Bolin, 1988; Zuerner, 1994). This element belongs to the recently recognized IS110 family of transposable elements (Zuerner, 1994). The genomes of different leptospiral serovars contain between zero and sixty copies of IS1533-related sequences (Zuerner & Bolin, 1990). Since the copy numbers differ in different serovars and among isolates of the same serovar, IS1533 is likely to be an active transposable element (Zuerner & Bolin, 1990; Zuerner *et al.*, 1993a; Zuerner, 1994). Analysis of the *L. borgpetersenii* serovar *hardjo* genome suggests that IS1533 may be involved in chromosomal rearrangements (Zuerner *et al.*, 1993a). Pulsed-field gel electrophoresis (PFGE) studies of *L. interrogans sensu lato* reveals extensive heterogeneity (Herrmann *et al.*, 1991, 1992) suggesting that this is a common phenomenon throughout this genus.

During construction of physical and genetic maps of *L. interrogans* serovars *icterohaemorrhagiae* strain Verdun (Baril *et al.*, 1992) and *pomona* strain RZ11 (Zuerner, 1991) several additional repetitive sequence elements were isolated, including an element related to the IS3 family of insertion elements. In this study we characterize copies of an IS3-like element from *L. interrogans*, and report its distribution in the genus *Leptospira*.

METHODS

Bacterial strains and plasmids. *Leptospira* strains (Table 1) were cultivated in EMJH medium (Ellinghausen & McCullough, 1965; Johnson & Harris, 1967) with agitation at 30 °C or 33 °C. Virulent *L. interrogans* strain Verdun was verified for virulence in guinea pigs, and its avirulent derivative was obtained by continuous *in vitro* subcultures. *Escherichia coli* strains XL1-Blue (Stratagene) and INVαF' (Invitrogen) were

cultivated in either Luria-Bertani (LB) or DYT media (Miller, 1972) at 37 °C. Plasmids pUC18, pCRII (Invitrogen) and pBluescript KSII+/- (Stratagene) were used as cloning vectors. *E. coli* containing recombinant plasmids was cultivated in the presence of either 100 µg ampicillin ml⁻¹ or 50 µg kanamycin ml⁻¹.

DNA electrophoresis and hybridization. Genomic DNA was prepared and separated by either continuous-field gel electrophoresis (CFGE) or PFGE using the protocols described previously by Baril *et al.* (1992), Zuerner (1991) and Zuerner *et al.* (1993b). Electrophoretically separated DNA was transferred to nylon membranes and used for hybridization analysis as described previously by Baril *et al.* (1992) and Zuerner (1991). Hybridization probes were prepared from cloned and uncloned PCR products or cloned genomic sequences. Probes were made with either digoxigenin-dUTP by random hexanucleotide priming reactions using a kit from Boehringer Mannheim, or [α -³²P]dATP (600 Ci mmol⁻¹, 22.2 TBq mmol⁻¹; ICN Pharmaceuticals) by nick translation using a kit from Gibco-Life Technologies. Visualization of hybridization results was done as described previously by Baril *et al.* (1992) and Zuerner (1991).

PCR. Three different sets of primers were used in PCR reactions to amplify *L. interrogans* DNA. The amplified DNAs were either used directly for probe synthesis or were first cloned into plasmid vectors. Primer set 1 (5'-ATT-CTA-AGT-TAG-TGT-GCA-3' and 5'-AGA-ATT-TCT-ATC-ACC-GAA-3') amplified between nucleotide 860 of IS1500 and a sequence downstream of IS1500. Primer set 2 (5'-AAA-GAA-GGA-CTC-AGC-GAC-TGC-G-3' and 5'-TTC-GAT-TCA-AAG-CAT-GGC-TAA-CG-3') amplified between 396 and 1056 of IS1500B (note that the primer starting at nucleotide 1056 encompasses a segment of sequence divergence between IS1500A and IS1500B). Primer set 3 (5'-ATG-GAA-ACT-CAT-CGA-TTT-GA-3' and 5'-TCG-ATC-CGA-ATG-AAA-AAC-TA-3') amplified between nucleotides 373 and 942 of IS1500. Amplifications using primer sets 1 and 3 were done in reaction mixtures containing 10 mM Tris/HCl, pH 8.4, 0.01% gelatin, 50 mM KCl, 1.5 mM MgCl₂, 200 µM dNTPs, approximately 5 ng of each primer µl⁻¹, and approximately 6 ng template µl⁻¹. Amplification reactions for primer set 2 were done in reaction

Table 1. *Leptospira* strains used in this study

Species	Serovar	Strain
<i>L. interrogans</i>	<i>icterohaemorrhagiae</i>	Verdun virulent
	<i>icterohaemorrhagiae</i>	Verdun avirulent
	<i>pomona</i>	RZ11
	<i>copenhageni</i>	M20
	<i>lai</i>	Lai
	<i>lora</i>	Lora
	<i>australis</i>	Ballico
<i>L. borgpetersenii</i>	<i>ballum</i>	Mus 127
<i>L. noguchii</i>	<i>louisiana</i>	LSU 1945
<i>L. weilii</i>	<i>celledoni</i>	Celledoni
<i>L. santarosai</i>	<i>sbermanii</i>	1342K
<i>L. kirschneri</i>	<i>grippotyphosa</i>	Moskva V
<i>L. inadai</i>	<i>ranarum</i>	Ranarum
<i>L. biflexa</i>	<i>patoc</i>	Patoc

Fig. 1. IS1500 nucleotide sequence analysis. (a) Structural features of IS1500. Selected restriction sites in IS1500, location and direction of ORFs, and IRs (arrowheads) are shown. The locations of the PCR-amplified DNA using three different primer sets are shown with boxes. (b) Nucleotide sequence of IS1500. The nucleotide sequences of IS1500A and IS1500B are shown, with homologous nucleotides in IS1500B shown as dots. The predicted amino acid sequences for ORFA and B are shown. Amino acid changes in the IS1500B ORFB sequence are shown in lower case next to the corresponding amino acid from the IS1500A ORFB sequence (which is shown in upper case). The putative translational shift sequences are blocked.

mixtures containing 10 mM Tris/HCl, pH 9.0, 0.1% Triton X-100, 50 mM KCl, 4 mM MgCl₂, 200 µM dNTPs, approximately 2.5 ng of each primer µl⁻¹, and approximately 6 ng template µl⁻¹. Reactions (50 µl or 100 µl vol.) were carried out either as described previously by Zuercher *et al.* (1993b) (primer set 2) or for 28 cycles of: 95 °C for 1 min; 44 °C for 1 min; and 72 °C for 3 min (primer set 1). Primer set 3 was used to amplify DNA using the same protocol as primer set 1 but with an annealing temperature of 50 °C, and 1.5 min extension at 72 °C. Probes synthesized from DNA amplified by primer sets 1 and 2 were used to screen genomic libraries for copies of IS1500. A probe synthesized from DNA amplified by primer set 3 was used to determine distribution, copy number and physical location of IS1500 insertions in the genome. The PCR products were cloned by ligation with *Sma*I-cleaved pUC18 DNA using the SureClone ligation kit (Pharmacia), or into pCRII using a kit

from Invitrogen. Ligations were transformed into *E. coli* strains XL1-Blue or INVαF' using standard techniques.

DNA sequencing and computer analysis. Nucleotide sequencing was done by dideoxynucleotide chain-termination reactions (Sanger *et al.*, 1977) on double-stranded templates with T7 DNA polymerase (Pharmacia or US Biochemical) in the presence of [³⁵S]dATPαS (Amersham). Reaction products were separated by electrophoresis through polyacrylamide-urea gels. Gels were either dried directly or first fixed with 10% (v/v) methanol/10% (v/v) acetic acid. Dried gels were used to expose XAR film (Kodak) at -80 °C. Nucleic acid and deduced amino acid sequences were analysed by BLAST (Altschul *et al.*, 1990), GCG (Genetics Computer Group), PC-GENE, Bionet (Intelligenetics), CLUSTAL (Higgins & Sharp, 1988) and PHYLIP version 3.5c (J. Felsenstein, Department of Genetics, University of Washington, Seattle) software, and by visual inspection.

(a)

IS1500A_A	MN-K-----	KGKYSPEFKEQAVKRTLSGS-FTI KEVAGSLGISYFVLRMRGEYLLKXSEDPLTDKQKSEELKLLKKEEVTILKX-----	FAANLS-----	REGNPD	99
IS1500B_A	MN-K-----	KGKYSPEFKEQAVKRTLSGS-FTI KEVAGSLGISYFVLRMRGEYLLKXSEDPLTDKQKSEELKLLKKEEVTILKX-----	FAANLS-----	REGNPD	99
IS1133_A	MS-L-----	K-H-SDEPKRDAVRIALTSG-LTRRQVADSLISGLSTLGM-----	IASISDETKIPTQDOLLKKEEVTILKX-----	FAVAKL-----	91
IS118_A	MS-G-----	K-RYPEEFKTEAVKQVVDLG-YSVASVATRLDITTHSLYAM-----	IKKYGPDSSTNKESQDAQAEIRRLKELKRVTDERDILKKAADVSDICONTFPNYLFRCFELOCLSRSVV		112
IS1206_A	M-P-----	RKTYTEEFKRAVALYENSPEASITQIATDLGVNRATLANVVKYGTG-APMETSPASVNEAEGIRKLKREHARLREERDILKKA		KYFA-----	97
ISL1_A	MTYS-----	RRRYDQDFKNVHSLFWSKSP-VKIIASELGPESALYRNKLYTE----	DGQGTFFASLEAH-BALKEHMEALAEHILKAA-----	AYFA-----	93
IS222_A	M-----	SKORRTFSAEFKREAAALVLDGG-YSHIDACRBLGVDSALRRVVKQLQAERGQVTPKSKALTPEQKKIOELKAIIRLEKALILKXATA-----		LLMSDEL-----	102
IS911_A	VICSPQNTGAPNKKRNFSAEFKRESAQLVDON-YTVADAANKVDGLSTMTNRVVKQLRDERGQTKPASPITPEQIETRELKCKLORIEMENEILKXATA-----			LLMSDSL-----	112

(b)

IS1500A_B	M-----	FMETHRFEYSIGGNHVLGVDSGFYFLKRSK--NELEKYNPELVEFIRETWLTSRKNYGLVRLLEVK-K-VYSIYGARTVRYKWLCEIGGQDEKR			96
IS1500B_B	M-----	FMETHRFEYSIGGNHVLGVDSGFYFLKRSK--NELEKYNPELVEFIRETWLTSRKNYGLVRLLEVK-K-VYSIYGARTVRYKWLCEIGGQDEKR			96
IS3_B	M-----	KTV-----	FIEKHQAEFSIKANCRVLRVARSQVYTCCORRRTISTROOFQHCOSVLAFTSRKQRYGAPRLTDELRAQ-GYFPNVTVAASLRQ-GLRAKASRK		101
IS600_B	M-----	CONFVSRSGYNNVQHP--SDRKOSDERLKEIKVANHRTREYTRRLLOTELAEN-GI-IVGDRRLARLRELRLCKQKRK			82
IS981_B	M-----	A-Q-----	TIQTAL--LNVRLSCOLLVPESSYIERINHP--SKTQLRRGYLSLKISQLFMANRGYGAPKIHMLLQK-GEKV-GLKLVKLMQQLKLSVVIKK		96
IS11_B	M-----	T-T-----	FIEKNSDQYPTKTLCDVLGIPRSTYKSLQHV--SHNRREKELTKKTIIEINTESKQRYGAPKIHFSLOKE-GYSV-SLKRVRRLKEAGIKSTVLKK		98
IS1206_B	VI-----	RFR-----	FVDDASKTYSVKRIICDVLKLRSSYKWKSTCSARRKRLMSDAILGARVKAFTTENGCGYGAIRITAEKLDQVDHDPVNHKRVARVNRSLKFGYTKKR		104
IS1163_B	XNVORIGTSSKGSIAKGGTRNFKSCGGVTGALGRINCLVFIEDGLLRSLIISLALKPRSTYNNKRYQP--SOHERVDOLKEKIKLWENNYRAYGYPRITMVLKSGIC--VGSKTILRLRENEIHSIMNR				136

IS1500A_B	FRIATDSSHNGHVRAPDLVORNFKPIQKNRIWSDITFLRS-SFGIYLCIILDYSRKVGWSISNSHNSKLVCTALSKATECRNPPKGLVFHSDRGSHYCSYETRYLLNNKLRRSHRKGCHDVAEVSFFGSLKR				235
IS1500B_B	FRIATDSSHNGHVRAPDLVORNFKPIQKNRIWSDITFLRS-SFGIYLCIILDYSRKVGWSISNSHNSKLVCTALSKATECRNPPKGLVFHSDRGSHYCSYETRYLLNNKLRRSHRKGCHDVAEVSFFGSLKR				235
IS3_B	FS-PVSYRANGLPVSENLLEQDFYASGNPKWAGDITTLRT-PEGMLYAVVIDLVSRAVIGWSNPRMTAGLADALOMALWRRKRPNNVINTDRGQYTSADYGAOLKRHLRGSSAKGCGYDNACVEVSFFHSLKV				229
IS600_B	FR-ATTNSHNLVPAPNLLMQTFAPAPNVAADLTIVAT-OEGMLYLAGIDVYTCEIVRYANGERMTKELTGKALFNALRSORPPAGLIHNSDRGSOYTCADYRVIGQSGIKTSNRKGYCHYDNAPHSFVGLKN				230
IS981_B	FKPGYSLSDHI-N-RKMLIQTEPTCK--MKVSTDIITYPT-OQGVASTLINDRYTKKVIAMOLGKMTVELVORTLNIKAISQDYEAVILNSDQSOYTSLEYEELIKYGYTHSFSRRGYPYNNASLESVMHGLKR				231
IS11_B	YHP-YSSKQGVVE-RENLEGNFSTKSIHEKMMADITYITLDRGNCYLASVLDLHNSKVVGSYFSRTITDVLKALENAYEAGPEEGLILHTLDRGSOYTSSEFAKPVSKGKQSFSGKGPYDNACIESFHAILKK				236
IS1206_B	KVTTTYSDDKTYVFPDLVGKRTFAMKPMVYVGDITVLPIDGSMYLATVDCYSRRLVGFSIADWRTSLVDOLAMAGORGLTGAIHNSDNGSVYTSNAPFOOTCDLGIROGMSIGTSADNALAESFMAALKR				243
IS1163_B	FKKPGTHVDHSG--RPNLIKHO----PHAIRURADITYLELRPGTVVYSSITYPKYHVLAFKIGRMEATLVETIHALEYHOKPQ--YFHSNGSOYTSNHEVETILERRHSHSNGKQGYTPDNGPEAFHSLKR				268

IS1500A_B	E-MEYNY-FYKIQEAEELLFDHIEVYNNRHSSSLDVSPVQFEVN-----AA				282
IS1500B_B	E-MEYNY-FYKIQEAEELLFDHIEVYNNRHSSSLDVSPVQFEVN-----AA				282
IS3_B	ECINGEN-FISREIMRATVFNIECDYNNRHSNCGGLSPEQFENK---HL-----A				288
IS600_B	ESLSHYR-FNNRDEAIVIREYIEIFYNRORRHSRLGHSAPAFREK---YHOMAA				272
IS981_B	EWYQFK-YKNFEAYQSI FMYIEAFYNSKRIGHSLGYLTPHQFE-K---V---SA				279
IS11_B	EEIHHVK-YLDFQEAQWALFYIEGYNNKRINGSIGYKTPQIE-D---NIHRTA				287
IS1206_B	EVLQDSKTFMHQLPCRRDVRNCTR-YNNVRRHSUCKYLAPAVFEKRCPAILKES				296
IS1163_B	E-FAQFTFSNFEDLVIRTSNYSI-WFNSDRIRTSV-----				302

Fig. 2. Comparison of IS1500-encoded proteins. (a) ORFAs from several IS3-like elements are compared. IS1500A-A and IS1500B-A refer to the ORFA proteins from IS1500A and IS1500B, respectively. (b) ORFBs from several IS3-like elements are compared. IS1500A-B and IS1500B-B refer to the ORFB proteins from IS1500A and IS1500B, respectively. Dashes (-) were inserted to optimize the alignments. Amino acids identical (*) or similar (Δ) to those in the IS1500-encoded proteins are shown below the sequences. The conserved D₃₅E motifs regions are underlined. Leucine zipper motifs (ORFA) and helix-turn-helix motifs (ORFB) in the IS1500-encoded proteins are in boldface type. Protein sequences were from: IS1B (Volkert *et al.*, 1994; accession number L20943), IS3 (Timmerman & Tu, 1985; accession number X02311), IS222 (Kropinski *et al.*, 1994; accession number U00100), IS600 (Matsutani *et al.*, 1987; accession number X05952), IS911 (Prere *et al.*, 1990; accession number X17613), IS981 (Polzin & McKay, 1991; accession number M33933), IS1133 (Chiou & Jones, 1993; accession number Z12167), IS1163 (Skaugen & Nes, 1994; accession number X75164), IS1206 (Bonamy *et al.*, 1994; accession number X69104), ISL1 (Shimizu-Kadota *et al.*, 1985; accession number X02734), and IS71 (accession number D10543).

RESULTS

Nucleotide sequence analysis

During earlier physical genomic mapping studies of *L. interrogans* (serovar *icterohaemorrhagiae* strain Verdun and serovar *pomona* strain RZ11) several cloned sequences were independently isolated and found to hybridize with multiple restriction fragments (Baril *et al.*, 1992; Zuerner, 1993; Zuerner *et al.*, 1993b). Cross-hybridization analysis showed that several different clones shared common sequences, suggesting that they were part of a related group of repetitive elements. Nucleotide sequence analysis showed the clones from strain Verdun contained sequences homologous to the 5' end of IS3 from *E. coli*, while the clones from strain RZ11 were homologous to the 3' end of IS3. Both sets of clones started at a conserved *Bam*HI site and extended in opposite directions from each

other. A composite sequence was generated by combining the sequences from strains Verdun and RZ11 at the *Bam*HI site, then designing PCR primers which could extend across this site. These primers directed amplification reactions, the products of which were used to screen genomic libraries from each strain for clones containing copies of the element. The nucleotide sequences from the genomic clones were determined.

One complete copy of the IS3-like repetitive element was characterized from each *L. interrogans* strain (Verdun and RZ11). The repetitive elements were 1236 bp in size and 98% identical in sequence. Both copies of the element have a DNA content of 35% G+C which is approximately the same as genomic DNA of *L. interrogans*. The termini of the elements from each strain contained imperfect inverted repeats (IRs) flanking 1159 bp

Fig. 3. Distribution of IS1500 in *Leptospira* species. An internal PCR-generated probe of IS1500 was used to hybridize with *Bgl*II-cut DNA from Verdun virulent (lane 1), RZ11 (lane 2), M20 (lane 3), Lai (lane 4), Lora (lane 5), Ballico (lane 6), LSU 1945 (lane 7), Celledoni (lane 8), 1342K (lane 9), Mus 127 (lane 10), Ranarum (lane 11), Moskva V (lane 12) and Patoc (lane 13) strains. The sizes (in kbp) and migration of standards are indicated.

'unique' regions (Fig. 1). These 'unique' regions contained four potential ORFs (*orfA-orfD*). Because the cloned elements have characteristic IS features they were designated IS1500, and called IS1500A (from strain Verdun) and IS1500B (from strain RZ11). The IRs are unequal in size with the left IR being 38 bp in length with the sequence 5'-TAA-CCT-AGT-TAC-GTA-AAA-ACG-GAC-ACC-TTT-TAC-TCA-GC-3', and the right IR being 39 bp in length with the sequence 5'-TAA-CCT-AGT-GAC-GAA-TTA-TTG-GAC-ACA-TTT-TTA-CGC-AGC-3'. Although the two copies of IS1500 shown share a cytosine immediately next to the right IR other copies of IS1500 do not (data not shown). Other sequences immediately next to the copies of IS1500A and B shown are different (Fig. 1) showing that the two copies are from different insertions in the leptospiral genome.

The IS1500 sequences were compared to the combined EMBL, PDB, GenBank, PIR, and SwissProt databases using BLAST and FASTA (GCG) algorithms. The 5' portion of IS1500 is approximately 70% similar to a 620 bp repetitive element characterized from *L. interrogans* serovar *hardjo* type *hardjoprajitno* described previously (Savio *et al.*, 1994; accession number X58829). IS1500 also shares sequence similarity to the IS3 family of transposable elements (see below).

Protein coding potential

The 'unique' region of IS1500 contains four ORFs. Starting from the left IR the first ORF (*orfA*) spans nucleotides 14–364 (using the coordinates in Fig. 1) and has the potential to encode a 11765 Da protein (starting with GTG at nucleotide 62). The second ORF (*orfB*) overlaps ORFA, spanning nucleotides 265 and 1212, is the largest, and has the potential to encode a 33355 Da protein (starting with ATG at nucleotide 367). There are two small ORFs on the complementary strand of IS1500: *orfC* (between nucleotides 1234 and 913) has the potential to encode a 10678 Da protein (starting with ATG at nucleotide 1171); and *orfD* (between nucleotides 420 and

227) has the potential to encode a 5908 Da protein (starting with ATG at nucleotide 384).

Searches of the protein and nucleotide databases using the BLAST and FASTA algorithms showed that the predicted ORFA and ORFB proteins were similar to the predicted proteins encoded by the IS3 family of transposable elements. Using the BLAST scores as a guide, the protein sequences most similar to the predicted IS1500-encoded ORFA and ORFB proteins were aligned using CLUSTAL and analysed with PHYLIP software. Those sequences found to be most alike to the IS1500 proteins are shown in Fig. 2. Searches of the databases failed to detect sequences with significant similarity to the ORFC or ORFD proteins.

Analysis of the predicted IS1500-encoded proteins using PROSITE suggested that ORFA has a leucine zipper motif (Fig. 2) common among many DNA-binding proteins (Landschulz *et al.*, 1988). Some ORFA proteins of other IS3-like elements also contain the leucine zipper motifs. ORFB has a helix–turn–helix motif (Fig. 2) which is also common to many DNA-binding proteins (Brennan & Matthews, 1989).

IS1500 copy number in *Leptospira* species

The minimum number of IS1500-like sequences in different members of the genus *Leptospira* was determined by Southern blot hybridization of CFGE-separated *Bgl*II-digested genomic DNA hybridized with an internal IS1500 probe (see Methods). Since the PCR-generated IS1500 probe did not span the *Bgl*II site in IS1500A or IS1500B, a single hybridizing fragment was assumed to represent a single copy of IS1500 or IS1500-like sequence. Fragments of varying intensity were detected (Fig. 3), and only the more intense bands were considered for determining approximate copy number. This approach may yield a conservative estimate of IS1500 copy number since more than one copy may reside on the same fragment or there may be two or more comigrating fragments which hybridize with the probe. There were at least eight copies

Fig. 4. Localization of IS1500 on the physical map of the *L. interrogans* genome. (a) Hybridization of an internal IS1500 probe with Verdun DNA digested with *NotI* (lane 1), *NotI* and *AscI* (lane 2), *AscI* (lane 3) and *SrfI* (lane 4). Sizes of the *NotI* fragments which hybridized with the probe are shown adjacent to lane 1. (b) Hybridization of clone KB4 with restriction-endonuclease-digested, PFGE-separated, RZ11 DNA. DNA was digested with *ApaI* (lane 1), *BssHII* (lane 2), *NotI* (lane 3), *SfiI* (lane 4) and *SmaI* (lane 5). Sizes of the *NotI* and a 40 kbp *ApaI* fragment which hybridized with the probe are shown adjacent to lane 5. (c) Comparison of IS1500 distribution in *L. interrogans* serovar *icterohaemorrhagiae* strain Verdun and serovar *pomona* strain RZ11. Approximate locations for IS1500 insertions in the genomes of strains RZ11 (top) and Verdun (bottom) are shown as blocks on the genetic map. Boxes indicating IS1500 insertions are shown as different sizes because they span the entire length of restriction fragments used for locating the insertion. For strain Verdun the presence of co-migrating fragments complicates the interpretation of the hybridization data, and probable (not confirmed) locations are shown as open boxes. Note that for strain Verdun part of the *NotI* B fragment is located at the left-hand side of the linearized physical map while the rest of this fragment is located at the right end, and likely constitutes a single insertion. Selected markers are shown. Approximate regions of the genome which are rearranged (inverted or having an insertion) between these two strains are shown by the lines between the two maps. The endpoints of the rearrangements are not known.

of IS1500 in *L. interrogans* strain Verdun (Fig. 3) and there were no differences between virulent strain Verdun and its avirulent derivative (data not shown). Strain RZ11 had about 11 copies of IS1500 (Fig. 3, and data not shown). There were at least 14 copies of IS1500 in both *L. noguchii* strain LSU 1945 and *L. kirschneri* strain Moskva V, six copies in *L. weilii* strain Celledoni, two copies in *L. santarosai* strain 1342K, and one copy each in *L. borgpetersenii* strain Mus 127 and *L. inadai* strain Ranarum (Fig. 3). No sequences hybridizing with the IS1500 probe were detected in *L. biflexa* (Fig. 3). Because some of the copies detected in *BglII* digests of leptospiral DNA were below the unit size of IS1500 it is likely that sequence heterogeneity resulting in 'new' *BglII* sites within the region spanned by the probe is present among different IS1500 or IS1500-like elements.

Localization of IS1500 on the physical maps of *L. interrogans*

Comparison of the physical and genetic maps of *L. interrogans* strains Verdun and RZ11 showed the presence of several large rearrangements which could be the result

of recombination between repetitive DNA (Zuerner *et al.*, 1993b). As a first step in assessing the possible role of IS1500 in generating some of these rearrangements we mapped the approximate location of IS1500 insertions on the physical maps of these two strains. The internal IS1500 probe was used to localize insertion sites in strain Verdun. This probe hybridized with *NotI* fragments A, B, C, F and H of strain Verdun (Fig. 4a). Because the *NotI* C, F and H bands each contain two fragments (C1, C2, F1, F2, H1 and H2) additional hybridization experiments were done with DNA digested with both *NotI* and *AscI*. Results of these experiments confirmed that copies of IS1500 are on the C1 and F2 fragments, and suggest that insertions may also be on the C2 and F1 fragments (Fig. 4a, and data not shown). Lack of suitable restriction sites in or near the *NotI* H1 and H2 fragments prevented us from determining if IS1500 is located on one or both fragments. Probes synthesized from cloned copies of IS1500B hybridized with *NotI* fragments A, B, C, E, F, H, and I of strain RZ11 (Fig. 4b, and data not shown). Further localization was made possible by following the hybridization patterns of the probe with *ApaI*, *BssHII* and *SmaI* (Fig. 4b) and *AscI*, *SgrAI*, *Sse8387I* and *SrfI*

fragments (data not shown). The approximate positions of IS1500 insertions in the genomes of strains Verdun and RZ11 are shown on the physical maps (Fig. 4c). It is important to note that these maps are the most conservative interpretation of the hybridization data allowing for only one copy per set of hybridizing restriction fragments. Thus, if two elements are closely placed on the same fragment they were counted as a single insertion.

DISCUSSION

Genetic organization in *L. interrogans sensu lato* is fluid. Closely related strains can be differentiated by large chromosomal rearrangements (Herrmann *et al.*, 1991, 1992; Zuerner *et al.*, 1993b). We have hypothesized that the prevalence of repetitive DNA, including IS-like elements, in the leptospiral genome contributes to the generation of these rearrangements (Zuerner *et al.*, 1993b). To understand better the potential involvement of repetitive DNA in generating these rearrangements we have initiated studies aimed at characterizing these elements. In this study we isolated and characterized different copies of an IS3-like element that we designated IS1500 from two closely related serovars of *L. interrogans sensu stricto*. This element has features characteristic of bacterial IS elements, including the presence of terminal IRs, and a central region containing ORFs. The lack of identical sequences immediately adjacent to the IRs suggests that IS1500 integrates without duplication of the target sequences as is often seen in other IS elements (Galas & Chandler, 1989). Lack of conservation among sequences flanking IS1500 suggests that this element lacks a highly conserved target site for integration. It is important to note that the 5' portion of IS1500 shares significant similarity to a 620 bp repetitive element cloned from *L. interrogans* serovar *hardjo* (Savio *et al.*, 1994; accession number X58829) suggesting that related IS3-like elements exist in the *L. interrogans* genome. Indeed, the presence of copies of this related element may account for variations in the hybridization signal seen in hybridization experiments with the IS1500-based probes (Fig. 3). The predicted IS1500-encoded proteins also share similarities to other IS3-like elements and to the D₃₅E superfamily of transposases (Doak *et al.*, 1994).

One hallmark of IS3-like transposable elements is the presence of overlapping ORFs encoding the putative transposases (Chandler & Fayet, 1993; Galas & Chandler, 1989; Polard *et al.*, 1991; Schwartz *et al.*, 1988). These ORFs typically start near the beginning of the central 'unique' region of the IS (*orfA*) and extend to very near the end of this region (*orfB*). In *E. coli*, IS3, IS150 and IS911 each encode three proteins of about 10000, 32000 and 42000 Da in size (Sekine *et al.*, 1994; Vögele *et al.*, 1991; Polard *et al.*, 1991). The two smaller proteins are products of *orfA* and *orfB*, respectively, with translation of *orfB* being coupled with translation of *orfA* (Sekine *et al.*, 1994). A (-1) translational shift fuses *orfA* with *orfB* when the ribosome encounters the sequence A₆G (IS150 and IS911) or A₄G (IS3) and yields the 42000 Da protein. It is the ORFAB fusion protein which is thought to be the functional transposase. Like IS3 and other members of the

IS3 family, IS1500 has an *orfA* and an *orfB*, encoding proteins of about 11000 and 33000 Da. Two potential translational shift sequences (A₅G and A₆G) occur in the *orfA-orfB* overlap (at nucleotides 265 and 364, respectively) and likely direct synthesis of an ORFAB fusion protein of about 45000 Da. Attempts to demonstrate synthesis of these proteins *in vitro* using cell-free extracts of *E. coli* have met with limited success; only the ORFA protein has been detected (data not shown).

The roles of the ORFA or ORFB proteins in the IS3 family of elements are not well understood. It has been suggested that the IS3 ORFA may repress transposition either by binding in a possible promoter for the IS3-encoded genes (and thus block transcription) or, alternatively, this protein may bind to the IRs (and thus block transposase from binding its target) (Sekine *et al.*, 1994). In contrast, the ORFA protein of IS911 appears to promote intermolecular transposition, but the mechanism by which this is achieved is unclear (Polard *et al.*, 1991, 1992). While these differences have yet to be resolved it is interesting to note that the ORFA proteins of different IS3-like elements are not as well conserved as the ORFB proteins (Schwartz *et al.*, 1988; Fig. 2). The IS1500 ORFA has a potential leucine zipper motif common among DNA-binding proteins (Landschulz *et al.*, 1988). Leucine zipper motifs are also seen among ORFA proteins from some, but not all, members of the IS3 family. Presence of a leucine zipper motif suggests that ORFA may interact with DNA as a dimer (Landschulz *et al.*, 1988). In IS1500 the leucine zipper motif is lost in the ORFAB fusion protein, suggesting that ORFA and the ORFA portion of the ORFAB fusion protein are functionally different. The IS1500 ORFB protein shares the D₃₅E motif which is also seen in eukaryotic retrotransposons (Doak *et al.*, 1994; Fayet *et al.*, 1990). It has been suggested that the D₃₅E motif has a functional role in catalysing part of the transposition reaction (Doak *et al.*, 1994). The ORFB protein and the ORFB-portion of the ORFAB fusion protein contain a helix-turn-helix motif suggesting both proteins interact with DNA (Brennan & Matthews, 1989). Further analysis is needed to define the functional regions of the IS-encoded proteins of the IS3 family.

Unfortunately, there is no known method to study genetic exchange in *L. interrogans*. Thus there are no data regarding the ability of these elements to transpose. Differences in IS1500 copy numbers in different *L. interrogans* serovars, and the presence of IS1500 in each of the known species of pathogenic leptospiral serovars, suggests that this element may be transpositionally active. Further study is needed to show transposition of IS1500.

One aspect of particular interest to our laboratories is the generation of chromosomal rearrangements in *L. interrogans*. Evidence for the presence of two chromosomal replicons (4.4–4.7 Mb, and 350 kb in size) in *L. interrogans sensu lato* has been presented (Zuerner *et al.*, 1993b). Comparison of genetic organization in two closely related serovars (*icterohaemorrhagiae* and *pomona*) shows that several chromosomal rearrangements have occurred in the large chromosomal replicons of these two serovars. Some of these rearrangements are inversions, spanning up to

34% of the large replicon. In several bacteria, including *E. coli*, regions of chromosomal instability can be mapped near IS elements (Birkenbihl & Vielmetter, 1990; Umeda & Ohtsubo, 1989). In *L. interrogans* serovars *icterohaemorrhagiae* and *pomona* strains Verdun and RZ11, all IS1500 insertions occur on the large chromosomal replicon (Fig. 4c), and many are located in regions of the chromosome containing rearrangements. Previously we found that IS1533-like sequences mapped near one of the rearranged segments of the chromosome (Zuerner *et al.*, 1993b). While these data favour a possible role of the repetitive elements in contributing to the genomic heterogeneity seen within *L. interrogans sensu lato*, the techniques used to localize the elements only provide a low resolution map and alternative elements or genetic mechanisms could also contribute to generating chromosomal rearrangements. No differences in the IS1500 hybridization patterns were detected between virulent and avirulent Verdun strains, suggesting that IS1500 rearrangement does not play a role in the loss of virulence of these strains. However, potential rearrangements involving other repetitive DNA species were not investigated. We are using information from this study to better localize the rearrangements and to determine if, or how, the rearrangements affect phenotype in *L. interrogans*.

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